Kidney International, Vol. 48 (1995), pp. 1031–1035

Molecular characterization of voltage and cyclic nucleotide-gated potassium channels in kidney

GARY V. DESIR

Section of Nephrology, Department of Medicine, Yale University School of Medicine and West Haven Veterans Administration Medical Center, New Haven, Connecticut, USA

Potassium (K^+) channels constitute a diverse group of membrane proteins that facilitate the passive movement (driven by the electrochemical gradient for K^+) of K^+ across cell membranes. One or more type of K^+ channel can be detected in virtually all mammalian cells. There are extensive data on the kinetic properties and the physiology of K^+ channels in kidney. More recently, the initial molecular characterization of some types of renal K channels has been reported. This paper will briefly review the potential physiologic role and the molecular biology of two types of renal K channels, namely those that are regulated by voltage and those that are regulated by cyclic nucleotide.

Voltage-gated renal K channels

Physiology of K_v

Potassium channels that open or close in response to changes in membrane voltage are called voltage-gated K^+ (K_v) channels. A subclass of K_v channels also require calcium for activation (maxi K^+ channels). Detailed kinetic analyses of K_v channels indicate that they constitute a heterogeneous class of transporters with differences in single channel conductance, in threshold for activation by voltage, in the kinetics of inactivation and in inhibitor profile. K_v channels have been studied most extensively in excitable cells where they participate in diverse cellular function such as action potential and pacemaker activity [1]. More recently, it has been appreciated that K_v channels may play a crucial role in the regulation of vascular smooth muscle contraction and therefore, peripheral vascular resistance and blood pressure [2–4]. In contrast, there are only few reports of K_v currents in epithelial cells (eye and type II pneumocytes) [5, 6], and until recently, K_v channels had not been detected in renal epithelia.

The primary physiological role of K_v channels in epithelial cell is uncertain for the following reasons. Most K_v channels have an activation threshold that is > -50 mV and open only when the cell membrane depolarizes beyond -50 mV. Such channels would remain closed in epithelial cells since the resting membrane potential of these cells remains between -60 and -90 mV under physiological conditions.

It is possible, however, that epithelial cells of the cortical collecting duct and of the renal medulla undergo changes in membrane potential substantial enough to activate K_v. In the collecting duct, transport of significant amount of Na^+ into the cell via the apical, amiloride-sensitive sodium channel would be expected to depolarize the membrane. In addition, sustained K^+ secretion in the lumen will increase extracellular K^+ concentration and cause the cell membrane to depolarize. Voltage and calcium-gated K^+ (maxi K^+) channels have been detected in rabbit cortical collecting duct [7]. They were initially thought to contribute significantly to K^+ secretion in the distal tubule, but it appears that under physiologic conditions these channels do not open very frequently and could not account for the observed rate of K^+ efflux.

In the inner medulla, extracellular K^+ can reach levels greater than 40 mM as a result of K^+ being trapped in the tubule by the well documented process of K^+ recycling [8]. The resting membrane potential is close to the reversal potential for K^+ (-90 mV) since K^+ channels constitute the major ionic pathway of unstimulated cells. Therefore, increasing extracellular K^+ from 4 to 30 mM will cause the resting membrane potential to change from approximately -90 mV to -30 mV. This degree of depolarization would be sufficient to activate many K_v channels since their threshold for activation is usually > -50 mV.

Recently, the first functional evidence of K_v expression in the renal medulla was provided by Volk et al [9] They found that in the rabbit papillary epithelial cell line GRB-PAP1, the predominant conductance was a slowly inactivating, time and voltage-dependent K current. The current activated at potential less negative than -30 mV; it was inhibited by barium, tetra ethyl ammonium (TEA) and 4-amino pyridine (4-AP). The kinetics characteristics and inhibitor profile of this K^+ channel are very similar to those of the voltage-gated K^+ channel proteins belonging to the *Shaker* superfamily. Indeed, The authors also provided preliminary molecular evidence for expression of a *Shaker*-like gene in these cells. These results corroborate previous work (to be discussed in more detail in the next section) carried out in our laboratory indicating that several *Shaker*-related K^+ channel genes are expressed in rabbit kidney.

Molecular biology of K_v

Shaker

It is beyond the scope of this paper to provide a comprehensive review of the molecular biology *Shaker* K_v channels. For that, the interested reader is referred to the recent review by Chandy and Gutman [10]. A gene family, discovered in *Drosophila* mutants six years ago and referred to as *Shaker*, encodes voltage-gated K

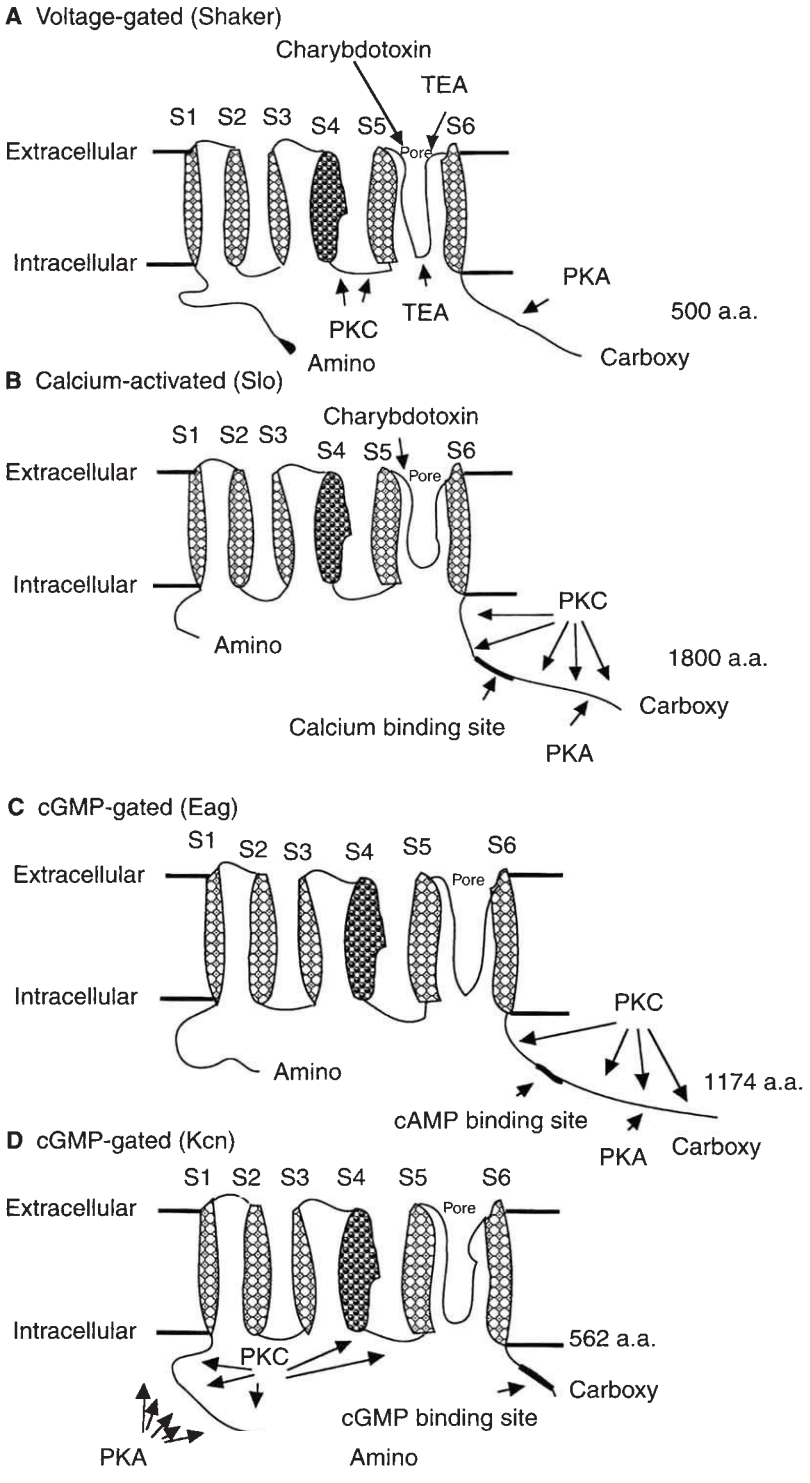

Fig. 1. *Proposed secondary structure of voltage and cyclic nucleotide-gated K^+ channels.* (**A**) Voltage-activated K^+ channel. Prototypic mammalian *Shaker* protein. S1-S6 denotes putative transmembrane segments. S4 is the voltage sensor. Binding sites for channels blockers are indicated. The pore region is located between S5 and S6. The amino terminus is involved in channel inactivation. Phosphorylation sites for protein kinase A (PKA) and C (PKC) are shown. (**B**) Calcium and voltage activated K^+ channel. The membrane topology of *Slo* is similar to that of *Shaker*, but overall amino acid identity is less than 20%. (**C**) cAMP-gated K^+ channel. Membrane topology also similar to that of *Shaker*. Amino acid identity is greater with non-selective cation channels than with *Shaker*. (**D**) cGMP-gated K^+ channel. Kcn has same membrane topology as *Shaker*. Amino acid identity with *Shaker* proteins ranges from 20 to 45%. It does not fit in the currently recognized *Shaker* subfamilies (Kv1 to 4.)

channels in vertebrates, insects and mollusks [11–20]. Most Shaker-related genes discovered to date arose from a common ancestor and fall into four well defined subfamilies (*Shaker*, Shab, Shaw and Shal) [21, 22]. The subfamilies are further subdivided: Shaker -Kv1.1 to 6; Shab-Kv2.1 to 2 and Kv5.1; Shaw -Kv3.1 to 4; Shal-Kv4.1 to 2 and Kv6.1 [23]. The deduced primary structure of the proteins indicate that they all contain six hydrophobic transmembrane segments (S1 to S6) (Fig. 1A) in addition to a pore region that traverses the plasma membrane twice. The fourth segment (S4) is an arginine-rich amphipathic helix that represents the voltage sensor. This segment consists of a repeated motif of a positively charged amino acid, arginine or lysine, followed by two hydrophobic residues. It is homologous to that found in the sodium and the dihydropyridine-sensitive calcium channels [22].

Structure-function relationships of *Shaker* proteins are being studied in great detail in naturally occurring isoforms and in mutants obtained by site-directed mutagenesis. It is clear that the S4 segment plays an important role in sensing the change in membrane voltage [24–27]. Regions involved in channel inactivation have been identified [28, 29]. Part of the pore of the channel is located between the 5th and 6th transmembrane segments [30–33]. This is also the region that binds the channel blockers TEA and charybdotoxin [34, 35]. *Shaker* channel activity is not only regulated by changes in membrane voltage but also by PKC dependent phosphorylation [36], serotonin, acetylcholine [32], and endothelin [20]. The great diversity of K_v currents detectable in cells can be explained by the large number of different *Shaker* proteins and by the fact that some native K_v channels are heteromultimers.

We recently cloned several K^+ channels isoforms from rabbit kidney and from two renal epithelial cell lines, LLC-PK_1 (pig proximal tubule) [37] using the polymerase chain reaction (PCR). There are at least six *Shaker* isoforms expressed in rabbit kidney and each isoform is encoded by a different gene. One of them, KC22, encodes a novel K^+ channel protein that is easily detected in rabbit kidney and in primary cell cultures of rabbit distal tubule [38]. We have proposed that KC22 may be involved in distal tubule transepithelial K transport. M1-CCD cells, derived from microdissected cortical collecting ducts of SV40 transgenic mice, express *Shaker* isoforms which are aldosterone-regulated [39].

SLOW

In *Drosophila*, mutations in the *slo* locus can alter or even completely eliminate maxi K currents. The mutation was mapped and the gene was recently cloned [40]. It encodes a protein with a membrane topology similar to *Shaker* channels: six transmembrane segments, a well conserved pore region and an S4 domain (Fig. 1B). In addition, it contains consensus phosphorylation sites for PKA and PKC, a potential ATP-binding site and a calcium-binding loop. Overall homology with *Shaker* proteins is about that 20%, indicating that this channel belongs to a related but distinct family of K channel proteins. Expression studies show that the *slo* protein form large conductance voltage-and calcium activated K^+ (maxi K) channels [41]. It is not yet known if renal maxi K channels are similar to *slo*.

IsK

The gene encoding a 17 kD protein (contains a single transmembrane domain) that is exclusively located on the luminal membrane of proximal tubule cells [42] was isolated in 1988 by Takumi, Ohkubo and Nakanishi [43] using the method of expression cloning. They argued that this protein IsK represented a new type of slowly activating voltage-gated K channel. Recent work indicates, however, that Isk may not form a channel, and that it may instead activate pre-existing K^+ and Cl^- channels [44]

Cyclic nucleotide-gated renal K channels (K_{nuc})

Physiology of K_{nuc}

The activity of cyclic nucleotide-gated K^+ channels is regulated by changes in intracellular cGMP and/or cAMP. By analogy with the non-selective cyclic nucleotide-regulated cation channels [45–47], a K^+ channel should be included in that category only if its contains functional binding sites for cyclic nucleotides.

K_{nuc} may play a role in transepithelial transport. Indeed, a voltage-gated cAMP regulated K channel was detected by patch clamping in A6 cells, an amphibian kidney cell line [48]. Channel activity was only seen in cells treated with aldosterone. A cGMP-gated K channel has not yet been described in epithelial cells although mesangial and LLC-PK_1 cells (pig kidney proximal tubule) produce significant amounts of cGMP [49]. Light et al have characterized a non-selective-cation channel that is inhibited by cGMP in the inner medullary collecting duct [50].

It is possible that intracellular production of cGMP in mesangial cell (derived from smooth muscle) could regulate their contractile state and affect the glomerular filtration rate by modulating K_{nuc} activity. Indeed, in some vascular beds, smooth muscle relaxation correlates with membrane hyperpolarization that is mediated in part by K^+ channels [51–53].

Molecular biology of K_{nuc}

Cyclic AMP-gated K channel

Mutations in the *ether a go-go (eag)* locus alter K^+ currents in *Drosophila* [54]. The *eag* gene was cloned and found to encode a membrane protein distantly related to *Shaker* and to the cyclic nucleotide-gated nonselective cation channels (Fig. 1C) [55]. When expressed in *Xenopus* oocytes, the *eag* protein forms K^+ and Ca^{2+} permeable channels that are directly activated by cAMP but not cGMP [56]. It is not yet known if the *eag* gene is expressed in the kidney.

Cyclic GMP-gated K channel

Since the molecular structure of cGMP-gated K channels was previously unknown we hypothesized that they might share structural motifs with both *Shaker* K^+ channels and cyclic nucleotide-gated cation channels. We screened a genomic rabbit library with a *Shaker* probe and a probe specific for cyclic nucleotide binding sites and isolated a gene encoding a novel protein (Kcn) that represents a cGMP-activated K channel [57]. This protein has six transmembrane segments, a voltage sensor (S4), a pore region and a cyclic nucleotide binding region (Fig. 1D). The regulation of channel function is likely to be complex since Kcn is not only activated by cGMP but also contains several putative phosphorylation sites for PKA and PKC and one for cGMP-dependent protein kinase. Furthermore, gene expression may be modulated by steroid hormones. This channel is expressed in kidney (tubules, glomeruli and arteries) and heart. We postulate that it may participate in transepithelial K transport, in the regulation of arterial tone. It may also modulate mesangial cell contractility and therefore GFR.

Conclusions

In the past six years our understanding of the molecular biology of potassium channels has grown tremendously. Some of the most extensively studied K channels are those that are voltage-gated (K_v). Although the physiological importance of K_v channels may not be immediately obvious in kidney epithelia, it is clear that several these genes are expressed in kidney and that K_v may play a role in K secretion in the cortical collecting duct and in K recycling in the inner medulla. Most recently the structure of cAMP and cGMP gated K channels has been described. These channels may participate not only in transepithelial K^+ transport but also in the regulation of vascular tone, and therefore modulate

GFR and blood pressure. Much work remains to be done to integrate our rapidly expanding knowledge of the molecular structures of these K^+ channels with the physiological data already available. It is also hoped that in some instances, the discovery of new molecular structures will expand the existing physiological framework and will lead to the development of new therapeutic agents.

Acknowledgments

The author was supported by Career Development and Merit Review awards, and a grant from the Veterans Administration, NIH (DK48105B).

Reprint requests to Gary V. Desir, M.D., Department of Medicine, Section of Nephrology, Yale University School of Medicine, West Haven VA Medical Center, 2073 LMP, 333 Cedar Street, New Haven Connecticut 06510, USA.

References

1. CATTERALL W, EPSTEIN PN: Ion channels. *Diabetologia* 35(Suppl 2):S23–S33, 1992
2. RUSCH NJ, STEKIEL WJ: Ionic channels of vascular smooth muscle in hypertension. *Adv Exp Med Biol* 308:1–7, 1991
3. SIEGEL G, EMDEN J, WENZEL K, MIRONNEAU J, STOCK G: Potassium channel activation in vascular smooth muscle. *Adv Exp Med Biol* 311:53–72, 1992
4. BOLOTINA VM, NAJIBI S, PALACINO JJ, PAGANO PJ, COHEN RA: Nitric oxide directly activates calcium-dependent potassium channels in vascular smooth muscle. *Nature* 368:850–856, 1994
5. RAE JL: Ion channels in ocular epithelia. *Invest Ophthalmol Vis Sci* 34:2608–2612, 1993
6. DECOURSEY TE, JACOBS ER, SILVER MR: Potassium currents in rat type II alveloar epithelial cells. *J Physiol* (Lond) 395:487–505, 1988
7. HUNTER M, LOPES AG, BOULPAEP E, GIEBISCH G: Regulation of single K channels from apical membranes of rabbit collecting tubules. *Am J Physiol* 25:F725–F733, 1986
8. JAMISON RL, WORK J, SCHAFER JA: New pathways for potassium transport in the kidney. *Am J Physiol* 242:F297–F312, 1982
9. VOLK KA, HUSTED RF, PRUCHNO CJ, STOKES JB: Functional and molecular evidence for Shaker-like K^+ channels in a rabbit papillary epithelial cell line. *Am J Physiol* (in press)
10. CHANDY GK, GUTMAN GA: Voltage-gated K^+ channels, in *CRC Handbook of Receptors and Channels*. (in press)
11. TEMPEL BL, PAPAZIAN DM, SCHWARZ TL, JAN YN, JAN LY: Sequence of a probable potassium channel component encoded at the *Shaker* locus of *Drosophila*. *Science* 237:770–775, 1987
12. TIMPE LC, SCHWARZ TL, TEMPEL BL, PAPAZIAN DM, JAN YN, JAN LY: Expression of functionnal potassium channels from *Shaker* cDNA in *Xenopus* oocytes. *Nature* 331:143–145, 1988
13. ATTALI B, HONORÉ E, LESAGE F, LAZDUNSKI M, BARHANIN J: Regulation of a major cloned voltage-gated K^+ channel from human T lymphocytes. *FEBS Lett* 303:229–232, 1992
14. FRECH GC, VANDONGEN AMJ, SCHUSTER G, BROWN AM, JOHO RH: A novel potassium channel with delayed rectifier properties isolated from rat brain by expression cloning. *Nature* 340:642–645, 1989
15. CHRISTIE MJ, ADELMAN JP, DOUGLASS J, NORTH RA: Expression of a cloned rat brain potassium channel in *Xenopus* oocytes. *Science* 244:221–224, 1989
16. BLAIR TA, ROBERDS SL, TAMKUN MM, HARTSHORNE RP: Functional characterization of RK5, a voltage-gated K^+ channel cloned from the rat cardiovascular system. *FEBS Lett* 295:211–213, 1991
17. KIRSCH GE, DREWE JA, VERMA S, BROWN AM, JOHO RH: Electrophysiological characterization of a new member of the RCK family of rat brain K^+ channels. *FEBS Lett* 278:55–60, 1991
18. RETTIG J, WUNDER F, STOCKER M, LICHTINGHAGEN R, MASTIAUX F, BECKH S, KUES W, PEDARZANI P, SCHRÖTER KH, RUPPERSBERG JP, VEH R, PONGS O: Characterization of a Shaw-related potassium channel family in rat brain. *EMBO J* 11:2473–2486, 1992
19. PO S, SNYDERS DJ, BAKER R, TAMKUN MM, BENNETT PB: Functional expression of an inactivating potassium channel cloned from human heart. *Circ Res* 71:732–736, 1992
20. ISHII K, NUNOKI K, MURAKOSHI H, TAIRA N: Cloning and modulation by endothelin-1 of rat cardiac K channel. *Biochem Biophys Res Commun* 184:1484–1489, 1992
21. BUTLER A, WEI A, BAKER K, SALKOFF L: A family of putative potassium channel genes in *Drosophila*. *Science* 243:943–947, 1989
22. JAN LY, JAN YN: Structural elements involved in specific K^+ channel functions. *Annu Rev Physiol* 54:537–555, 1992
23. GUTMAN GA, CHANDY GK: Nomemclature of mammalian voltage-dependent potassium channel genes. *Semin Neurosci* 5:101–106, 1993
24. BEZANILLA K, PEROZO E, PAPAZIAN DM, STEFANI E: Molecular basis of gating charge immobolization in *Skaker* potassium channels. *Science* 254:679–683, 1991
25. LIMAN ER, HESS P, WEAVER F, KOREN G: Voltage-sensing residues in the S4 region of a mammalian K^+ channel. *Nature* 353:752–756, 1991
26. MCCORMACK K, TANOUYE MA, IVERSON LE, LIN JW, RAMASWAMI M, MCCORMACK T, CAMPANELLI JT, MATHEW MK, RUDY B: A role for hydrophobic residues in the voltage-dependent gating of *Shaker* K^+ channels. *Proc Natl Acad Sci USA* 88:2931–2935, 1991
27. PAPAZIAN DM, TIMPE LC, JAN YN, JAN LY: Alteration of voltage-dependence of *Shaker* potassium channel by mutations in the S4 sequence. *Nature* 349:305–310, 1991
28. HOSHI T, ZAGOTTA WN, ALDRICH RW: Biophysical and molecular mechanisms of Shaker potassium channel inactivation (see comments). *Science* 250:533–538, 1990
29. ZAGOTTA WN, HOSHI T, ALDRICH RW: Restoration of inactivation in mutants of Shaker potassium channels by a peptide derived from ShB (see comments). *Science* 250:568–571, 1990
30. PONGS O: Structural basis of voltage-gated K^+ channel pharmacology. *Trends Pharmacol Sci* 13:359–365, 1992
31. HEGINBOTHAM L, ABRAMSON T, MACKINNON R: A functional connection between the pores of distantly related ion channels as revealed by mutant K^+ channels. *Science* 258:1152–1155, 1992
32. HARTMANN HA, KIRSCH GE, DREWE JA, TAGLIALATELA M, JOHO RH, BROWN AM: Exchange of conduction pathways between two related K^+ channels. *Science* 251:942–945, 1991
33. YOOL AJ, SCHWARZ TL: Alteration of ionic selectivity of a K^+ channel by mutation of the H5 region. *Nature* 349:700–704, 1991
34. MACKINNON R, MILLER C: Mutant potassium channels with altered binding of charybdotoxin, a pore-blocking peptide inhibitor. *Science* 245:1382–1385, 1989
35. GOLDSTEIN SAN, MILLER C: A point mutation in a *Shaker* K^+ channel changes its charybdotoxin binding site from low to high affinity. *Biophys J* 62:5–7, 1992
36. ISACOFF EY, JAN YN, JAN LY: Putative receptor for the cytoplasmic inactivation gate in the *Shaker* K^+ channel. *Nature* 353:86–90, 1991
37. DESIR GV, HAMLIN HA, PUENTE E, REILLY RF, HILDEBRANDT F, IGARASHI P: Isolation of putative voltage-gated epithelial K-channel isoforms from rabbit kidney and LLC-PK_1 cells. *Am J Physiol* (Renal Fluid Electrol Physiol) 262:F151–F157, 1992
38. DESIR GV, VELAZQUEZ H: Identification of a novel K-channel gene (KC22) that is highly expressed in distal tubule of rabbit kidney. *Am J Physiol* 264:F128–F133, 1993
39. DESIR GV: Molecular physiology of renal potassium channels. *Semin Nephrol* 12:531–540, 1992
40. ATKINSON NS, ROBERTSON GA, GANETZKY B: A component of calcium-activated potassium channels encoded by the *Drosophila slo* locus. *Science* 253:551–555, 1991
41. ADELMAN JP, SHEN KZ, KAVANAUGH MP, WARREN RA, WU YN, LAGRUTTA A, BOND CT, NORTH RA: Calcium-activated potassium channels expressed from cloned complementary DNAs. *Neuron* 9:209–216, 1992
42. SUGIMOTO T, TANABE Y, SHIGEMOTO R, IWAI M, TAKUMI T, OHKUBO H, NAKANISHI SJ: Molecular cloning and sequence analysis of human genomic DNA encoding a novel membrane protein which exhibits a slowly activating potassium channel activity. *Biochem Biophys Res Commun* 161:176–181, 1989
43. TAKUMI T, OHKUBO H, NAKANISHI S: Cloning of a membrane protein that induces a slow voltage-gated potassium current. *Science* 242: 1042–1045, 1988
44. ATTALI B, GUILLEMARE E, LESAGE F, HONORE E, ROMEY G, LAZDUNSKI M, BARHANIN J: The protein IsK ia a dula activator of K^+ and Cl^- channels. *Nature* 365:850–852, 1993
45. FILATOV GN, JAINAZAROV AB, KOLESNIKOV SS, LYUBARSKY AL,

FESENKO EE: The effect of ATP, GTP and cAMP on the cGMP-dependent conductance of the fragments from frog rod plasma membrane. *FEBS Lett* 245:185–188, 1989
46. FURMAN RE, TANAKA JC: Photoreceptor channel activation: Interaction between cAMP and cGMP. *Biochemistry* 28:2785–2788, 1989
47. MICHEL WC, ACHE BW: Cyclic nucleotides mediate an odor-evoked potassium conductance in lobster olfactory receptor cells. *J Neurosci* 12:3979–3984, 1992
48. HAMILTON KL, EATON DC: cAMP-induced potassium channel activity in apical membrane of cultured A6 kidney cells. *Am J Physiol* (Renal Fluid Electrol Physiol) 261:F1055–F1062, 1991
49. ISHII K, CHANG B, KERWIN JF: Formation of endothelium-derived relaxing factor in porcine kidney epithelila LLC-PK^1: An intra- and inter-cellular messenger for activation of soluble guanylate cyclase. *J Pharmacol Exp Ther* 256:38–43, 1991
50. LIGHT DB, SCHWIEBERT EM, KARLSON KH, STANTON BA: Atrial natriuretic peptide inhibits a cation channel in renal inner medullary collecting duct cells. *Science* 243:383–385, 1989
51. PALMER RMJ, FERRIGE AG, MONACADA S: Nitric oxide release accounts for the biological activity of endothelium-derived relaxing factor. *Nature* 327:524–526, 1987
52. NELSON MT, PATLAK JB, WORLEY JF, STANDEN NB: Calcium channels, potassium channels, and voltage dependence of arterial smooth muscle tone. *Am J Physiol* 259:C3–18, 1990
53. STANDEN NB, QUAYLE JM, DAVIES NW, BRAYDEN JE, HUANG Y, NELSON MT: Hyperpolarizing vasodilators activate ATP-sensitive K^+ channels in arterial smooth muscle. *Science* 245:177–180, 1989
54. ZHONG Y, WU CF: Alteration of four identified K^+ currents in *Drosophila* muscle by mutation in *eag*. *Science* 252:1562–1564, 1991
55. GUY RH, WARMKE J, DRYSDALE R, GANETZKY B: Similarities in amino acid sequences of *Drosophila eag* and cyclic nucleotide-gated channels. *Science* 254:730, 1991
56. BRÜGGEMANN A, PARDO LA, STUHMER W, PONGS O: Ether-à-go-go encodes a voltage-gated channel permeable to K^+ and Ca^{2+} and modulated by cAMP. *Nature* 365:445–448, 1993
57. DESIR GV, WELLING PA, ENGEL D, ZHANG J: Molecular cloning and expression of a cyclic nucleotide-gated K^+ channel. *PNAS* (in press)